The Effects of Surface-Applied Jasmonic and Salicylic Acids on Caterpillar Growth and Damage to Tomato Plants[1]

Aaron L. Iverson, Louis R. Iverson, and Steve Eshita, Buckeye Valley High Schools, Delaware, OH 43015 and USDA Forest Service, Delaware, OH 43015

Abstract. We tested the role of salicylic acid (SA) and jasmonic acid (JA) in altering the tomato plant's defense against herbivory by tobacco hornworm. Treatments of SA or JA were topically applied to tomato plants, hornworm consumption was allowed to proceed for 12 days, and harvest analyses were performed. Measurements taken included a subjective plant rating (1-10 score), plant dry mass, caterpillar mass, and the number of times the caterpillars fell off the plant. Results showed significant effects of exogenously applied SA and JA on the defense of tomato plants against insect herbivory. Plants treated with SA had little resistance to the feeding caterpillars and the plant lost more biomass to them. JA, in contrast, apparently increased the defensive mechanisms of the plant, resulting in lower caterpillar growth and increased caterpillar detachment from plants. The data are consistent with a model where JA, endogenous or exogenously applied, is necessary for defense against insect herbivory and SA disrupts JA biosynthesis and/or pool accumulation.

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INTRODUCTION

Jasmonic acid (JA) is an endogenous plant growth regulator widely distributed in higher plants (Meyer and others 1984; Tizio 1996). In response to injury, a plant may produce JA, which induces the expression of defensive compounds such as insect proteinase inhibitors. JA may also be systemically distributed throughout the plant and create volatile gases, which in turn may induce neighboring plants to increase their defense allocations as well as attract parasitic wasps to attack the infesting herbivores (Creelman and Mullet 1995; McConn and others 1997; Thaler and others 1996; Turlings and others 1995). The synthesis of jasmonic acid takes place via the octadecanoid pathway (Fig. 1). The precursor of jasmonic acid is linolenic acid. Linolenic acid is converted to hydroperoxylenolenic acid by lipoxygenase. After reactions catalyzed by allene oxide synthase (AOS) and allene oxide cyclase, phytodienoic acid is formed and through oxidation, jasmonic acid is formed (Creelman and Mullet 1997; Pan and others 1998). The jasmonic acid then facilitates the induction of plant defensive genes.

Salicylates, when synthesized or applied to plants, inhibit AOS activity, which in turn inhibits the production of jasmonic acid and proteinase inhibitors (Fig. 1) (Raskin 1992; Doares and others 1995; Pan and others 1998). Consequently, a plant given salicylates will become less able to defend itself against insect attack. In contrast, elevated SA in plants has often been associated with increased pathogen resistance (Yang and others 1997). However, the relationship between pathogen and insect resistance is still under debate (Apriyanto and Potter 1990; Hatcher 1995). Some studies show mutual antagonism of JA vs. SA pathways, with consequent increase in pathogen resistance but decrease in insect resistance with the exogenous application of SA, while

Linolenic Acid
↓ *13-lipoxgenase*
Hydroperoxy-octadecatrienoic Acid
SA block → → ↓ *allene oxide synthase (AOS)*
Allene Oxide
↓ *allene oxide cylcase (AOC)*
12-Oxo-phytodienoic Acid (PDA)
↓ *12-oxo-PDA reductase, β oxidation*
7-epi-Jasmonic Acid (JA)
↓
Gene Expression

Figure 1. Pathway of jasmonic acid systhesis in plants, also showing the point of inhibition by salicylic acid in the pathway (after Pan and others 1998).

others have shown cross protection for both insect and pathogen resistance (for example, Inbar and others 1998; Thaler and others 1999). The plant response apparently depends on the plant-challenger system and the type and strength of the elicitor.

Several investigations have centered on the defense of the tomato (*Lycopersicon esculentum*) and their insect pests. Howe and others (1996) found that a tomato mutant that was deficient in the capacity to induce defense genes, via the octadecanoid pathway, was much more susceptible to damage by the tobacco hornworm (*Manduca sexta*). Thaler and others (1996) found that exogenously applied JA increased defense against beet armyworm (*Spodoptera exigua*), and showed that field-applied JA enhances the production of chemical defenses in tomato. Thaler (1999a) also showed that JA, when exogenously applied to tomato, not only induced additional plant resistance to beet armyworm damage, but also doubled the incidence of parasitism of the endoparasitic wasp *Hyposoter exigua* on the armyworm. These results may indicate a potential use of JA in inhibiting agricultural pests.

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In this experiment, we investigate the tomato-hornworm system, a well-studied and economically important crop-insect pest system. We test the effect of surface-applied salicylic acid (SA) and jasmonic acid (JA) on tomato plants, in conjunction with the damage induced by herbivory by the tobacco hornworm.

METHODS

Plant Growth

Heirloom tomato seedlings (*Lycopersicon esculentum* (L.) Mill., cv. Moskovich) were germinated under fluorescent lights (17h:7h, L:D, 21° C) in seed starters and transplanted as 16-day old seedlings into 10 cm pots and placed in a greenhouse (sodium lights; 17h:7: L:D; 24-29° C photophase and 16-18° C scotophase). Plants were allowed to grow for 12 days following transplantation before chemical or caterpillar treatments were applied. Plants were randomly placed in rows according to each treatment. Each row of six replicate plants was separated from other rows (treatments) by at least 60 cm. This spacing was necessary to reduce possible effects of spreading volatile gases or caterpillars among the various treatments. A total of 24 plants were given caterpillar treatments, with six replicates each of four surface-applied treatments (SA, JA, both, or water). An additional 18 plants, to serve as controls for caterpillars, were not given caterpillars but three surface-applied treatments (SA, JA, or water). Pots within rows and rows themselves were shuffled every 2-3 days to account for any unequal illumination of light among the plants. The plants were evaluated daily and watered as needed throughout the experiment.

Phytohormone Application

Solutions of 0.01% JA (methyl jasmonate, IUPAC name: (-)-1α,2β-3-Oxo-2-(cis-2-pentenyl)-cyclopentaneacetic acid methyl ester, obtained as a gift from R.A. Creelman) and 0.05% SA (methyl salicylate, IUPAC name: 2-hydroxybenzoic acid methyl ester; obtained from Sigma Chemical Co., St. Louis, MO) were prepared for the chemical treatments. Concentration levels were chosen based on previous work by Browse (Browse, personal communication). First, stock solutions of SA (5%) and JA (1%) were prepared with ethanol, as was a control with neither SA nor JA. Ten ml of each stock solution was then added to 990 ml of deionized water to obtain the desired concentrations of 0.05% for SA, 0.01% for JA, and control. In each of the three solutions, six drops of Tween 20 (detergent, obtained from R.A. Creelman) were added to allow equal distribution of liquid on the plant leaf surface and to allow the plant to absorb the solution more readily (Creelman, personal communication; Browse, personal communication).

The plants were sprayed with hand spray bottles two times—one day prior and one hour prior—to the application of the caterpillars. Each plant was sprayed with a fine mist of 2-2.5 ml of SA, JA, control, or both SA and JA solutions. The solutions had dried prior to initial application of the caterpillars. Plants were then sprayed every two days over a 12-day period until the experiment was terminated.

Caterpillar Treatment

Tobacco hornworm (*Manduca sexta*) eggs were obtained from the North Carolina State University Insectary. Eggs were placed in a 25 × 25 cm plastic container with artificial diet at room temperature and in the natural light regime for January in Ohio. The artificial diet was obtained also from the NCSU Insectary and consisted of a mixture of wheat germ, casein, sucrose, torula yeast, Wesson salt mixture, sorbic acid, cholesterol, methyl paraben, streptomycin sulphate, agar, vitamin mixture (USB no. 23430), ascorbic acid, and formalin. Eggs hatched in 4-6 days. Four 1- to 2-day old larvae were placed on each caterpillar-treatment plant and allowed to consume foliage for 12 days. Caterpillars were counted daily on each treatment plant; frequently some caterpillars had detached from the plant to the soil surface of the pot or table just below the plant. These caterpillars were tallied as 'detached' and replaced.

Analysis Of Treatment Effects

The plants were subjectively scored beginning the day after the treatments were applied and continuing every two days until the day of harvest. The levels of rating were as follows:

1 = No damage, perfectly healthy
2 = Slight leaf discoloration or chlorosis
3 = Slight leaf wilting or curling
4 = More leaf curling or wilting
5 = Some leaves curled or wilted over half
6 = Some leaves curled or wilted over three-fourths
7 = All leaves curled or wilted over half
8 = All leaves curled or wilted full
9 = Dead leaves
10 = Plant dead

After the 12 days, the 6-week old plants were cut at the base, placed in a paper bag, dried for 48 hours in a drying oven at 70° C, and weighed (in bags) with a Fisher top-loading balance accurate to 0.01 g. Because the 18 caterpillar-control plants also served as controls for another experiment testing the effects of UV-C light in conjunction with SA and JA (not reported here but which experiment was terminated 4 days earlier), these control plants were harvested 4 days earlier than the rest of the experiment. Therefore, one would expect those plants to have slightly less dry weight than they would be if allowed to grow as long as the caterpillar-applied plants. Those data are included in some of the analyses because of the additional information obtained. Data of score, plant weight, caterpillar weight, and number of caterpillar detachments were statistically analyzed in S-Plus software (Statistical Sciences 1993) using an analysis of variance (ANOVA) with multiple comparisons tested with the Tukey method. The P value used for declaring significant effects was 0.05.

RESULTS

Plant Scores

Plants with no caterpillars, regardless of chemical treatment were vigorous and healthy throughout the experiment (score of 1; Table 1). Thus, the JA and SA did

TABLE 1

Effects of surface-applied JA and SA on tomato plant vigor and weight.

Variable Treatment	Caterpillars added				No Caterpillars added (Control)[1]		
	Water	SA	JA	SA+JA	Water	SA	JA
Mean plant vigor score	3.70 ± .28	4.03 ± .27	3.20 ± .20	3.57 ± .15	1.0 ± 0	1.0 ± 0	1.0 ± 0
Mean plant weight, g[2]	0.63 ± .09 b	0.41 ± .14 a	0.82 ± .11 c	0.69 ± .08 b	0.97 ± .11 x	1.16 ± .12 y	0.93 ± .11 x

[1] Because the plants with no caterpillars were harvested 4 days earlier, only general comparisons should be made to the caterpillar-treated plants.
[2] Different letters indicate significantly different (P <0.05) results using Tukey's multiple comparison test after the analysis of variance.

not impact plant vigor. With caterpillar treatment, all plants suffered damage, with the SA-treated plants having greater damage as compared to controls, both, and especially the JA treatment.

Plant Weight

Plants treated with caterpillars had generally lower biomass compared to plants with no caterpillars (Table 1). This effect of a reduction in plant biomass via caterpillar ingestion is apparent even though the control plants that had no caterpillars applied were harvested four days earlier than the caterpillar-treated plants.

Among the plants treated with caterpillars, aboveground dry matter data showed that the SA-treated plants had significantly lower total yield compared to water-treated plants, while JA-treated plants had higher yields compared to water-treated plants and especially when compared to SA-treated plants (Table 1). JA-treated plants had twice the biomass of the SA-treated plants. When SA and JA were applied together, there was a slight, but not statistically different, increase in biomass compared to controls (0.69 ± .08 vs. 0.63 ± .09 g per plant). In general, the effects of JA vs. SA negated each other. Conceivably, exogenous JA should be able to bypass and eventually overcome the SA block on endogenous JA production, if enough exogenous JA was applied and absorbed. Perhaps this effect is beginning to be apparent in this experiment.

Among the controls that had no caterpillars added, SA-treated plants had a significantly higher biomass relative to the JA- or water-treated plants (Table 1). Further, the JA-treated plants had a slightly, but statistically insignificant, lower mean compared to the water-treated plants. These trends are opposite to those observed when caterpillars were added to the plants.

Caterpillar Weight

Caterpillars on SA-treated plants grew 55% larger than those on controls, and 221% larger than those grown on JA-treated plants (Table 2). In contrast, caterpillars grown on JA-treated plants showed a 52% reduction in growth compared to controls, while those grown on plants treated with both SA and JA had a 43% reduction in growth compared to controls. This trend is consistent with exogenous JA overcoming the SA block of JA biosynthesis.

Caterpillar Detachment

This metric is a measure of the total number of times caterpillars had dropped from the plant to the pot or table, cumulative over the 12 days of caterpillar consumption. Though no statistical analysis was possible on these data, the JA-treated plants had nearly twice the number of detachments compared to water-treated plants, and three times the detachments of SA-treated plants (Table 2). Those plants treated with both JA and SA had nearly the same number of detachments as compared to the JA-treated plants. These data suggest that caterpillars were making choices on preferred food, and the JA-treated plants were not preferred. Because the plants were widely spaced within the greenhouse, no evidence of crossover of caterpillars between treatments was detected.

DISCUSSION

Evidence from plant weight, caterpillar weight, and the cumulative count of caterpillar detachments shows that the surface application of SA made tomato plants more susceptible to caterpillar damage and that JA made tomato plants less susceptible to caterpillar damage.

TABLE 2

Effects of surface-applied JA and SA on caterpillar weights and detachments.

Treatment	Water	SA	JA	JA+SA
Mean caterpillar weight, mg[1]	87 ± 33 b	135 ± 36 c	42 ± 8 a	53 ± 16 ab
Total Detachments	17	11	31	30

[1] Different letters indicate significantly different (P <0.05) results using Tukey's multiple comparison test after the analysis of variance.

SA-treated plants had proportionately more plant tissue converted to caterpillar larvae tissue than did JA-treated plants. In both plant and caterpillar weight, SA and JA-treated plants were significantly different from the control treatment, but in opposite directions.

Plants treated with salicylic acid (SA) apparently have a diminished resistance to caterpillar damage. This trend is consistent with a model where SA blocks the production of allene oxide synthase (AOS), which is a necessary enzyme to produce jasmonic acid (JA) through the octadecanoid pathway (Fig. 1). JA is one of the signals for the plant to produce a defensive reaction against insect attack. With its defense impaired, a SA-influenced plant is unable to produce the necessary defensive compounds such as polyphenol oxidase (as well as peroxidase and lipoxygenase) or proteinase inhibitors (digestibility reducers) and becomes more susceptible to the herbivore (Stout and others 1998a, 1998b; Fidantsef and others 1999). As a result, the plant is consumed at a faster rate and consequently has a smaller final mass than control plants. Thus, caterpillars feeding on SA plants have a larger mass as they are not as inhibited by the proteinase inhibitors or other defense responses that can affect the growth and reproduction of the insect.

Plants treated with JA, on the other hand, exhibit the opposite effect. JA is the end product of the octadecanoid defense pathway that leads to the activation of defense genes and production of defensive proteins. In our model, exogenous JA is absorbed and stimulates a greater production of these defensive compounds and makes the plant more resistant than a control plant with fewer activated defensive compounds. Caterpillars feeding on JA-treated plants will, therefore, have a smaller mass than caterpillars feeding on control plants. As such, the JA-treated plant is also consumed at a slower rate and has a greater mass than the controls.

A large effort is underway to find and test suitable chemicals for field application that promote varying degrees of plant protection (Inbar and others 1998). JA may be a suitable candidate for insect control in agriculture. No negative effects on crop yield have been found, and plant resistance is enhanced both by directly killing herbivores and by enhancing the action of natural enemies of herbivores after JA application in field tests by Thaler (1999a, 1999b). However, the cost of JA treatment may be prohibitive at this time.

When evaluating the results for the plants not treated with caterpillars, the significantly higher biomass for SA-treated plants relative to JA- or water-treated plants was unexpected and not fully understood. Perhaps there is a physiological explanation but more research is needed. One speculation is that the plant, when treated with SA, has reduced photosynthate allocated to the production of secondary compounds such as jasmonic acid, so that more photosynthate is available for allocation to biomass. In the absence of a caterpillar attack, the plant fares better. However, if the plant is attacked, there are serious costs to the plant by not producing sufficient secondary metabolites such as JA. This process is termed the 'allocation model', described by Herms and Mattson (1992) and generally accepted by the community, but some recent work does not support this model (for example, Agrawal and others 1999). A second speculation could be that the additional SA could lead to an increased systemic resistance to a pathogen that might have been present in the plants, thereby allowing better growth.

General Interpretations

This study provides further evidence of the elaborate chemical communication and defense systems of plants. Surface application of JA adds resistance to herbivory, a trait which holds great potential application in agriculture to aid in pest management. Although not assayed in this experiment, the surface-applied JA apparently enhances the production of secondary metabolites via the octadecanoid pathway, so that additional defensive compounds could be produced.

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